

# Pathways for self-tolerance and the treatment of autoimmune diseases

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Antigen delivers both immunogenic and tolerogenic signals to lymphocytes. The outcome of antigen exposure represents a complex integration of the timing of antigen binding with signals from many other immunogenic and tolerogenic costimulatory pathways. A road map of these signalling pathways is only beginning to be charted, revealing the mechanism of action and limitations of current immunotherapeutic agents and the points of attack for new agents. Cyclosporin and tacrolimus interfere with tolerogenic signals from antigen in addition to blocking immunogenic signals, thus preventing active establishment of tolerance. Corticosteroids inhibit a key immunogenic pathway, NF $\kappa$ B, and more specific inhibitors of this pathway may allow tolerance to be actively established while immune responses are blocked. New experimental therapies aim to mimic tolerogenic antigen signals by chronically stimulating antigen receptors with antigen or antibodies to the receptor, or aim to block costimulatory pathways involving CD40 ligand, B7, or interleukin 2. Obtaining the desired response with these strategies is unpredictable because many of these signals have both tolerogenic and immunogenic roles. The cause of autoimmune diseases has been determined for several rare monogenic disorders, revealing inherited deficiencies in tolerogenic costimulatory pathways such as FAS. Common autoimmune disorders may have a biochemically related pathogenesis.

Self-tolerance is an essential feature of the immune system, and works to protect tissue antigens from becoming targets of damaging immune responses during clearance of infection. The immune system normally exhibits exquisite specificity in distinguishing infectious antigens from self antigens. Vigorous antibody or T-cell responses are mounted against infectious antigens, whereas self antigens generally elicit only transient or weak responses even when incorporated into an infectious particle.

Adaptive immune responses start with the binding of antigen to antigen receptors on rare lymphocytes. The number and activity of these cells is then greatly expanded by clonal proliferation and differentiation. The response of individual lymphocytes is governed, however, by opposing immunogenic and tolerogenic signals, and the latter normally prevail for lymphocytes that bind self antigens. Disturbance in the natural balance between immunogenic and tolerogenic signals due to genetic factors can give rise to autoimmune disease. Progress in delineating these opposing signals provides opportunities to correct the primary disorder in autoimmune patients.

## Counterbalancing immunogenic and tolerogenic signals

Two basic types of extracellular stimuli control lymphocyte growth and development (figure 1). The first is antigen signalling, through clone-specific antigen receptors. The second is costimuli, which encompasses a number of signals, through receptors that are not antigen specific. Importantly, particular antigen or costimuli

signals are rarely obligately immunogenic or tolerogenic. Their timing and the way they are integrated at key checkpoints in lymphocyte development determines how a lymphocyte responds. Strongly immunogenic costimuli can shift the balance to immunity in the face of strongly tolerogenic antigen signals, and strongly tolerogenic costimuli can over-ride strongly immunogenic antigen signals. Deciphering the molecular logic behind this signal integration is the central challenge facing clinical manipulation of tolerance and immunity.

## Immunogenic and tolerogenic antigen signals

Antigens transmit signals to lymphocytes by binding to B-cell receptors (surface immunoglobulin on B cells), and to T-cell receptors (TCRs) on T cells. B-cell receptors and TCRs signal through a cascade of protein tyrosine kinases and protein-lipid phosphorylation. Antigen transmits immunogenic or tolerogenic signals to lymphocytes through these receptors. Continuous

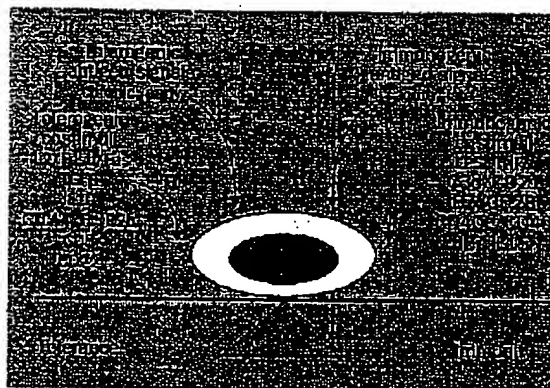
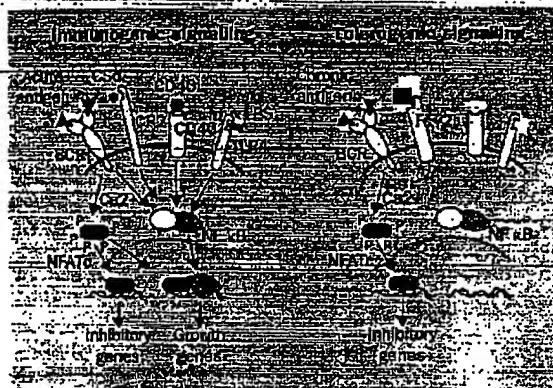


Figure 1: Schematic diagram illustrating the balance of immunogenic and tolerogenic signals affecting lymphocyte responses to antigen. LPS=lipopolysaccharide.

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**Figure 2: Biochemically distinct signals underpin immunogenic and tolerogenic responses to antigen in B lymphocytes**

Immunogenic signalling occurs when antigen is encountered suddenly by mature B cells, and is augmented by co-clustering of complement C3d-receptor, CR2, and concurrent stimulation by CD40L from helper T cells or bacterial products such as lipopolysaccharide (LPS). One of the chief pathways activated by these signals is NFκB, a DNA binding protein family that moves to the nucleus once activated. In the nucleus, NFκB is pivotal to stimulating expression of many key B lymphocyte growth genes, promoting cell proliferation and antibody. The B-cell receptor also activates another DNA binding protein, NFATc, which moves to the nucleus after calcium-induced dephosphorylation and can work synergistically with NFκB. On its own, NFATc can activate inhibitory genes such as the inhibitory receptor CD72. Tolerogenic signalling occurs when antigen is encountered chronically, which results in inhibitory changes that diminish calcium signalling so that NFκB is no longer activated. Co-clustering of the receptor for IgG, FcγR2b, also inhibits immunogenic signalling to NFκB. Absence of costimuli such as CD40L or LPS is also critical to allow tolerogenic signalling to proceed in the absence of NFκB.

binding of antigen over several days, as is often the case for self antigens, usually transmits tolerogenic signals. By contrast, a sudden increase in receptor crosslinking, as occurs in most infections, tends to transmit immunogenic signals. Binding of antigen during immature lymphocyte formation in bone marrow or thymus, as occurs for many self antigens but few infectious antigens, tends to be tolerogenic.<sup>1</sup> Immunogenic signals are favoured when antigen is first encountered after lymphocytes have matured and reached the secondary lymphoid tissues, where infectious antigens tend to be trapped.

Immunogenic and tolerogenic antigens elicit different biochemical signals within lymphocytes<sup>2</sup> (figure 2). These biochemical differences provide opportunities to develop immunosuppressants that mirror these different signal patterns. In mature B lymphocytes, tolerogenic signalling by antigen elicits a smaller calcium response than immunogenic antigen. The calcium concentration achieved with tolerogenic signals is enough to activate the nuclear factor of activated T cells (NFATc) but insufficient to activate the nuclear factor kappa binding molecule (NFκB). NFATc and NFκB are DNA binding transcription factors that promote expression of different sets of genes. NFAT is essential for turning on lymphocyte inhibition as well as activatory genes, whereas NFκB is more purely immunogenic, because it is essential for inducing genes necessary for B and T cell proliferation and antibody production. As a result, a different pattern of gene expression is established by tolerogenic and immunogenic exposures to the same antigen.<sup>3</sup>

Deficiency of the NFκB transcription factor, c-rel, abolishes both T and B cells' immunogenic responses to antigen.<sup>4</sup> The inherited immunodeficiency syndrome, X-linked agammaglobulinaemia, is caused by defects in Bruton's tyrosine kinase (BTK), an intracellular enzyme

that is essential for immunogenic signalling to NFκB by B-cell receptors.<sup>5</sup> Tolerogenic signalling to antigen remains intact or enhanced in BTK-defective B cells. This selective role in immunogenic signalling might explain the powerful suppression of systemic lupus in NZB/W mice when defects in BTK are introduced by breeding.<sup>6</sup> The selective role of the BTK/NFκB pathway in immunogenic signalling to antigen thus makes it an attractive target for new immunosuppressive drugs.

#### *Immunogenic costimuli from microorganisms*

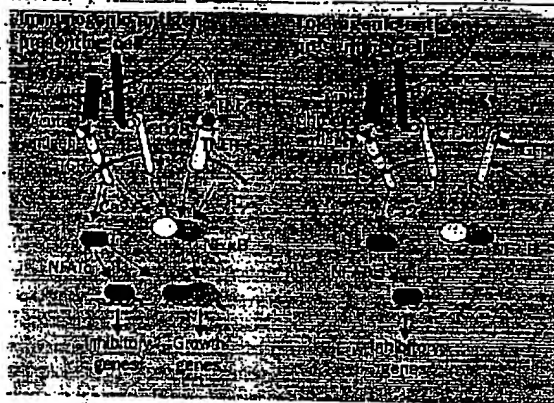
Costimuli arise from many sources in the lymphocyte microenvironment. Perhaps the only purely immunogenic costimuli come from conserved components of infectious microorganisms. The lipopolysaccharide (LPS) moiety of bacterial cell walls and DNA rich in the dinucleotide, CpG from bacteria both activate the NFκB pathway in lymphocytes through surface receptors of the Toll-like receptor (TLR) family<sup>7</sup> (figure 2). These immunogenic costimuli also signal lymphocytes indirectly by activating antigen presenting cells—dendritic cells, macrophages, and B cells, to produce additional immunogenic costimuli such as the T cell activating cell surface protein B7 (CD80) and the inflammatory cytokine tumour necrosis factor alpha (TNFα). Bacterial adjuvants have been explored as experimental therapeutics to promote immunogenic responses to autoantigens on tumour cells but give rise to other undesirable inflammatory effects. Their effect may be more specifically emulated by activating dendritic cells bearing tumour antigens in vitro and giving these cells to the patient.

#### *Costimuli from stressed and dying cells*

Cell death through apoptosis occurs physiologically in healthy tissues without inflammation or immunogenicity. Engulfment of apoptotic cells by tissue macrophages, dendritic cells, or fibroblasts elicits signals through the phosphatidylserine receptor that promote synthesis of the tolerogenic cytokine, transforming growth factor beta (TGFβ; figure 3) and inhibit production of the immunogenic cytokine TNFα.<sup>8</sup> By contrast, pathological cell death by necrosis links antigens with immunogenic costimuli. Necrotic cells, and antigens released from necrotic or stressed cells complexed with the heat-shock proteins, Hsp96 and Hsp70, activate dendritic cells to express immunogenic costimuli including B7 and TNFα.<sup>10-12</sup> In patients and animals models with developing neoplasms, increased production of these immunogenic costimuli through cell dysplasia and necrosis may account for the frequent detection of subclinical autoantibodies and for the less frequent paraneoplastic autoimmune syndromes. The latter might simply reflect rare clinical manifestations of common autoimmune responses to dysplastic tumour cell autoantigens, as a result of chance reactivity of the autoantibodies with a vital cell receptor. Likewise, cell stress and dysfunction in specific organs, such as the pancreatic beta cell, may be an immunogenic costimulus for autoimmunity.

#### *Dual role of the complement system*

Activation of the serum complement system by foreign cells or particles produces powerfully immunogenic costimuli, partly by covalently tagging the infectious antigens with the complement cleavage product C3d.<sup>13</sup> C3d signals immunogenically to B lymphocytes, through the complement C3d receptors, CR1 and CR2 (CD21



**Figure 3: Biochemically distinct signals underpin immunogenic and tolerogenic responses to antigen in T lymphocytes**

Immunogenic signalling occurs when antigen peptide and MHC complexes are encountered suddenly by mature T cells, and is augmented by concurrent stimulation by B7 molecules engaging CD28, or binding of TNF $\alpha$ . One of the chief pathways activated by these signals is NF $\kappa$ B, a DNA binding protein family that moves to the nucleus once activated. In the nucleus, NF $\kappa$ B is pivotal to stimulating expression of many key T lymphocyte growth genes, promoting cell proliferation and inflammatory cytokines. The TCR also activates another DNA binding protein, NFATc1, which moves to the nucleus after calcium-induced dephosphorylation and can work synergistically with NF $\kappa$ B. On its own, NFATc1 can activate inhibitory genes such as the death receptor ligand, FasL. Tolerogenic signalling occurs when antigen is encountered chronically, which results in inhibitory changes that diminish calcium signalling so that NF $\kappa$ B is no longer activated, CD28 is downregulated and an inhibitory receptor for B7, CTLA4, is upregulated. Concurrent stimulation by TGF $\beta$  inhibits expression of lymphocyte growth genes.

and CD35), when a C3d-tagged antigen causes clustering of these receptors with the B-cell receptors (figure 2). Complement components C1, C2, C4, and the CR1/2 complement receptors are also important for delivering tolerogenic signals, since inherited deficiencies of these elements in human beings and mice are associated with susceptibility to autoimmune disease. C1q deficiency leads to an inability to clear apoptotic cells efficiently, and this may either diminish the tolerogenic signals elicited by physiological cell corpses or allow them to become immunogenic.<sup>14</sup>

#### Dual role of the B7 system

Cell surface proteins of the B7 family, displayed on antigen presenting cells such as macrophages, dendritic cells, and B lymphocytes, deliver immunogenic costimuli to T cells by signalling through the CD28 and inducible costimulator (ICOS) receptors<sup>15,16</sup> (figure 3). The B7.1 and B7.2 proteins are induced on antigen presenting cells by other immunogenic costimuli, such as LPS, necrotic cells, or immunogenic antigen receptor signals in B cells, creating a cascade of immunogenic signals. Immunosuppressive therapy aimed at blocking the immunogenic effects of B7.1 and B7.2, notably the recombinant protein antagonist CTLA4-Ig, has been shown to improve the symptoms of psoriasis.

B7/CD28 costimuli are tolerogenic in other contexts, notably in immature thymocytes where they enhance clonal deletion. The B7/CD28 pathway also promotes tolerance by signalling the formation of regulatory CD4<sup>+</sup>CD25<sup>+</sup> T cells that may be required for tolerance to tissue antigens.<sup>17</sup> B7.1 and B7.2 proteins also transmit tolerogenic signals to T cells by engaging another receptor, CTLA4, that is present at very low levels in resting T cells and substantially increased by chronic antigen signals<sup>18</sup> (figure 3). The importance of CTLA4 as a brake to the system is shown by the lethal inflammatory

and lymphoproliferative disorder that occurs in CTLA4-deficient mice, and by the augmented autoimmune responses to melanoma antigens that occur when CTLA4 is blocked with antibodies.

#### Dual role of TNF $\alpha$ family of proteins and receptors

Activation of T cells and other innate or adaptive immune cells elicits an important and growing class of immunogenic and tolerogenic costimuli related to the cytokine, TNF $\alpha$ . TNF $\alpha$  itself has a pleiotropic effect on immune responses and inflammatory cells.<sup>19-21</sup> In some contexts, TNF $\alpha$  promotes self-tolerance and CD8 T cell deletion, whereas in others TNF $\alpha$  promotes T cell activation and autoimmune disease. Inherited deficiencies in TNF $\alpha$  or its receptors in mice results in poor cytotoxic T-cell-mediated resistance to certain viruses and inability to form follicular dendritic cells needed for humoral immunity. Symptoms of rheumatoid arthritis improve after blocking TNF $\alpha$  with antibodies or recombinant protein antagonists, indicating that production of this cytokine by T cells in the synovium has a key inflammatory role.<sup>22</sup>

CD40-ligand (CD40L) and Fas-ligand are two proteins related to TNF $\alpha$  with essential regulatory functions. Both are membrane-bound proteins displayed on T cells following T-cell receptor signals. CD40L engages its receptor, CD40, on B cells and dendritic cells to activate immunogenic responses through the NF $\kappa$ B pathway.<sup>23</sup> The importance of CD40L as an immunogenic costimulus is shown in children and mice with inherited CD40L deficiency, the X-linked hyper-IgM syndrome, where there is an absence of IgG antibody responses and defective T-cell immunity. Experimental therapies based on blocking the immunogenic effects of CD40L on B cells and dendritic cells with antibodies showed spectacular promise in animal models, notably achieving long-term allograft tolerance in primates.<sup>24</sup> Clinical trials in human beings have been suspended, however, because of thromboembolic complications in a subset of participants.

CD40L also seems to have an important tolerogenic role, since CD40L-deficient children are also commonly affected by autoimmune disease. CD40L is needed as a tolerogenic signal for B cells to increase expression of Fas (CD95), the receptor for FAS-L.<sup>25</sup> FAS itself transmits a potent tolerogenic costimulus by triggering the death and deletion of self-reactive B and T lymphocytes. The importance of the FAS pathway is seen by the systemic Autoimmune Lymphoproliferative Syndrome (ALPS) in human beings and mice with inherited deficiencies in FAS-L, FAS, or the downstream protease Caspase-10.<sup>21,26</sup>

#### Inhibitory co-receptors

This is a rapidly growing class of receptors that transmit inhibitory or tolerogenic costimuli to lymphocytes functions by recruiting protein tyrosine or lipid phosphatases. The prototype for this family is the low affinity receptor for IgG, Fc $\gamma$ R2b, on B cells.<sup>27</sup> Antigen and antibody complexes cause the antigen receptors to cluster with Fc $\gamma$ R2b, preventing B cell activation by otherwise immunogenic antigens and, instead, triggering death and deletion of the B cells (figure 2). This mechanism is believed to explain the tolerogenic effect of anti-RhD prophylaxis, in which small amounts of IgG anti-RhD antibodies given to Rh-negative mothers prevent maternal antibody responses to fetal RhD antigen.

### Cytokines

Cytokines deliver both immunogenic and tolerogenic costimuli to lymphocytes. This balance is well illustrated by interleukins 2, 7, and 15.<sup>21,22-25</sup> All three signal T and B cells through multisubunit receptors that share a common gamma chain ( $\gamma c$ ). Inherited deficiency of the  $\gamma c$  subunit accounts for X-linked Severe Combined Immunodeficiency, characterised by lack of naïve or memory T and B cells. IL-7 delivers essential costimuli through  $\gamma c$  that promote naïve T and B cell formation in thymus and bone marrow and promote survival of naïve T cells in the lymph nodes. Similarly, growth and persistence of memory CD8 T cells is promoted primarily by IL-15. By contrast, the essential function of IL-2 *in vivo* is to deliver a tolerogenic costimulus, despite its original discovery in tissue culture as a so-called T-cell growth factor. Mice lacking IL-2 or the unique IL-2 receptor alpha subunit develop a severe T cell lymphoproliferative disease with numerous autoantibodies. IL-2 sensitises T cells to receive tolerogenic signals by the Fas receptor system, and may also be required to sustain a tolerogenic subset of CD4+CD25+ regulatory T cells.

Transforming growth factor beta (TGF- $\beta$ ) delivers an important tolerogenic signal to lymphocytes, and mice lacking this cytokine rapidly develop a lethal syndrome of lymphocyte hyperactivity and autoantibodies.<sup>26</sup> TGF- $\beta$  inhibits the entry of lymphocytes into the cell cycle, and thus might establish a high tolerogenic threshold against which immunogenic signals from antigen and costimuli must work to initiate lymphocyte responses. The early response to immunogenic antigen is differentiated from tolerogenic antigen responses in part by rapid downregulation of inhibitory transcription factors in the former.<sup>27</sup> TGF- $\beta$  seems likely to establish these inhibitory factors in quiescent and tolerised lymphocytes. TGF- $\beta$  production by macrophages is induced by recognition and engulfment of cells that have died by physiological (non-inflammatory) apoptosis.<sup>28</sup> Macrophages, dendritic cells, and T cells making TGF- $\beta$  seem to promote tolerance to self and foreign antigens in the eye, lung, and gut.<sup>29,30</sup> Linking antigen signals with TGF- $\beta$  signals may be the basis for the experimental phenomenon of oral tolerance. Clinical trials are underway aimed at preventing type 1 diabetes or ameliorating multiple sclerosis by inducing oral tolerance to pro-insulin or myelin basic protein.

### Integration of tolerogenic and immunogenic signals at different steps in the immune response

Integration and timing of antigen signals and costimuli occur at numerous checkpoints in lymphocyte development. These checkpoints are placed all along the developmental pathway, from those that delete newly formed B or T cells in the bone marrow and thymus through to those that abort the formation of terminally differentiated plasma cells or killer cells. Lymphocytes integrate antigen signals and costimuli very differently from one checkpoint to another, because expression of receptors and their intracellular response machinery change during development. The multiplicity of checkpoints exists presumably for two main reasons. First, no single mechanism can adequately ensure tolerance to all self antigens. Second, the existence of multiple mechanisms balances the need for tolerance against the need to use cells that crossreact between self and foreign antigens for rapid immunity against infection.<sup>31</sup>

### Clonal deletion in central lymphoid tissues

In the bone marrow and thymus, antigens that cluster antigen receptors rapidly and avidly—which would be immunogenic for a mature lymphocyte—are almost exclusively tolerogenic for newly-formed B and T cells.<sup>32</sup> The basis for the tolerogenic response of immature lymphocytes seems to be a result of many things: differences in the second messengers elicited by antigen receptors in immature cells, differences in the set of genes that can be triggered by second messengers, and presence of tolerogenic costimuli in the bone marrow and thymus microenvironments. Immature thymocytes are triggered to die even when antigen signals are linked with costimuli such as B7/CD28 that would be immunogenic to mature T cells. In immature B cells, continuous B-cell receptor engagement with strongly crosslinking self antigens, such as DNA or surface antigens on haematopoietic cells, delivers a tolerogenic signal that immediately arrests the cell's maturation and leads to clonal deletion within 1–3 days. Some of these arrested cells reach the spleen before dying, but they are extraordinarily refractory or anergic to immunogenic costimuli such as LPS and CD40. Particular combinations of immunogenic costimuli, such as CD40 and IL-4 from helper T cells, may be able to over-ride the powerfully tolerogenic signals from self antigen in these situations and break tolerance at this point.

Only a subset of self antigens are nevertheless present in sufficient quantity in the thymus and bone marrow to trigger clonal deletion. There is simply not enough antigen to signal deletion for most clones which recognise antigens present in trace quantities in the circulation or which are restricted to other tissues, such as the pancreatic islets, the brain, or the thyroid. Other mechanisms normally ensure tolerance to these antigens.

### Clonal anergy

Self antigens that are present in lesser amounts in the bone marrow or thymus, or that cluster antigen receptors less avidly, can signal repeatedly to B and T cells without attaining the threshold needed to trigger arrest and death.<sup>33</sup> This constant "tickling" of antigen receptors by self antigens nevertheless transmits tolerogenic signals, activating feedback mechanisms that render the cell more refractory or anergic to immunogenic antigen signals. Anergy mediates B cell tolerance to self DNA and chromatin, and CD4 T cell tolerance to systemic and organ-specific antigens. In both B and T cells, anergy seems to involve a selective weakening of the connections between antigen receptors and the NF $\kappa$ B and JNK intracellular signalling pathways. Signalling through other intracellular pathways such as NFAT remains intact, so that a different set of tolerogenic genes is induced and immunogenic cell growth genes controlled by NF $\kappa$ B and JNK are not called into action. The weakening of connections to NF $\kappa$ B and JNK raises the threshold of immunogenic signalling needed to trip a self-reactive cell into multiplication. In B cells, a sudden burst of very avid antigen receptor clustering, or strong signals from LPS or CD40, allow sufficient signalling to the NF $\kappa$ B pathway to break anergy and drive the cell growth cycle.

### Clonal deletion and regulation in peripheral lymphoid tissues

In addition to anergy, a series of peripheral deletion mechanisms catch self-reactive cells that reach the spleen, lymph nodes, and other organs.<sup>34</sup> These peripheral tolerance checkpoints act by shortening

lymphocyte lifespan, inhibiting lymphocyte migration and recirculation, or causing rapid cell death in germinal centres or liver. These peripheral processes are for the most part poorly understood in biochemical terms, with the exception of the peripheral elimination of autoreactive B and T cells through the Fas cell death pathway.<sup>21,22,23,27</sup>

### Pathogenesis of autoimmune diseases

How does autoimmune disease arise? Given the range of self-tolerance processes, and the difficulty eliciting or maintaining autoimmune responses by deliberate means (for example in medical and veterinary efforts to achieve immunological contraception or castration), it is reasonable to ask how tolerance to one or more self antigens fails in many people. The reason is as yet unknown, except for the rare patients with inherited monogenic disorders such as ALPS and X-linked hyper-IgM.

Most of the common autoimmune diseases also have an important inherited element, contributing as much as 50% of the population risk, and particular types of autoimmune diseases thus cluster in families. This inherited susceptibility is nevertheless complex involving combinations of many different gene alleles.<sup>28</sup> The strongest contributions are made by particular haplotypes of the major histocompatibility complex (MHC) and specific HLA alleles within the MHC, whose products present antigen peptides to T cells. Exactly how particular MHC alleles predispose to autoimmunity is not yet established, and one can hypothesise too much or too little presentation of particular antigens by products of susceptible HLA alleles. Correlations between autoimmune susceptibility and many other chromosomal regions have been found in human beings and mice, but the complexity of the inheritance pattern has made it challenging to identify the non-MHC susceptibility genes.

Four basic kinds of defect may potentially give rise to autoimmune disease, either alone or in combination. A central challenge for clinical immunology will be to define which of these faults actually applies for individual patients, since the nature of the deficit will determine the success or failure of emerging therapeutic strategies.

#### *Insufficient tolerogenic signalling from antigen*

In order for deletion, anergy, or regulation to be triggered by tolerogenic signalling through antigen receptors, a sufficient number of receptors must be engaged on self-reactive cells. Autoantigens that are only present in trace amounts in the lymphatic tissues will not achieve this signalling threshold on any but the very highest affinity clones. If the autoantigen is highly expressed in extralymphatic sites, as is the case for insulin, thyroglobulin, myelin proteins, skin basement proteins, and type 2 collagen, these concentrated depots of autoantigen might suddenly deliver an acute immunogenic stimulus to self-reactive cells that chance to migrate into these sites. This situation seems to be the case for B cells and some CD8 T cells.<sup>29,30</sup> For CD4 T cells recognising such antigens, there seems to be some autoantigen encountered in lymphatic sites that might induce anergy and regulatory cells.<sup>31</sup>

Several susceptibility genes for type 1 diabetes may act by further diminishing this already limiting pathway for tolerogenic autoantigen presentation. Diabetes-susceptible MHC Class II alleles in human beings and mice seem less efficient at presenting antigens, potentially explaining the heightened risk of autoimmunity in individuals who are homozygous for these alleles.<sup>32</sup> A

variant allele of this insubergenic associated with type 1 diabetes susceptibility is expressed at lower levels in the thymus, potentially lessening presentation and education of regulatory T cells to this antigen.<sup>33</sup>

If the primary lesion in individuals susceptible to type 1 diabetes and other organ-specific diseases is simply one of inadequate tolerogenic signals from the target self antigens, then delivering more of these antigens in a tolerogenic form is a rational strategy. Obviously, this approach has the risk of inducing autoimmunity if the self antigen is delivered in an immunogenic form in some individuals, either due to the way the antigen is presented, to presence of immunogenic costimuli, or to presence of primed or memory lymphocytes that may be more refractory to tolerogenic signals. A better understanding of the molecular integration of tolerogenic and immunogenic signals may be critical to the success of specific vaccines against diabetes and other autoimmune diseases.

Insufficient tolerogenic signals from autoantigen might also explain shortcomings of the immunosuppressive drugs, ciclosporin and tacrolimus (FK506). These drugs block the calcium/calcineurin/NFAT signalling pathway. This pathway is continually activated by self antigen in anergic B and T cells, and is important for inducing tolerogenic costimuli on lymphocytes such as CD72 and FAS-L. Interference with these actively tolerogenic signals might explain the systemic autoimmune disorders that can occur after cessation of the drug, and might account for the inability to achieve long-term allograft acceptance with these agents.<sup>34</sup> The presence of circulating autoantibodies may compound autoimmunity in systemic lupus by blocking the presentation of tolerising autoantigens to B cells.<sup>35</sup>

#### *Too much immunogenic signalling from antigen*

Sudden presentation of viral or bacterial antigen in a highly crosslinked, immunogenic form, and associated with immunogenic costimuli produced by the infection, can provoke immune responses from T or B cells that crossreact with the microbial antigen and a self antigen. In animal models, this route can activate ignorant T and B cell clones that, through a combination of lower affinity receptors and limiting self-antigen presentation, had not received appreciable tolerogenic signals.<sup>36,37,38,39</sup> Moreover, if the immunogenic antigen stimulus is very strong, such as occurs with highly multimeric forms of antigen for B cells, the stimulus can overcome strongly tolerogenic antigen signals to break anergy<sup>40,41</sup> or over-ride clonal deletion.<sup>42</sup> Whereas a microbial trigger is postulated to be the cause of a number of common autoimmune diseases, perhaps the best established clinical example is the immunopathological damage of heart valves by antibodies that crossreact between valvular antigens and streptococcal M protein.

Interestingly, the self-reactive components of crossreactive responses are usually transient and lack memory in most experimental and practical situations in which tolerance is transiently broken by immunogenic delivery of self and foreign antigens. This phenomenon is a longstanding problem for medical and veterinary efforts to achieve immunocontraception and immuno-castration, in which the autoantibody titres to pregnancy or sex hormones fall prematurely in the face of heightened titres to the foreign carrier proteins. Susceptibility to full-blown autoimmune disease might therefore require that a crossreactive trigger be coupled with deficits in the tolerogenic costimuli that normally create an inhibitory feedback on self antigen responses.



### Deficiency of tolerogenic costimuli

Many of the rare systemic autoimmune disorders that are inherited as monogenic traits in human beings and mice arise from deficiencies of tolerogenic costimuli. The clearest example is human autoimmune lymphoproliferative syndrome (ALPS), which results from partial or complete deficiency in signalling by the death receptor FAS.<sup>21</sup> Similarly, deficiency of CD40L in X-linked hyper-IgM syndrome is commonly accompanied by autoimmune disorders that might reflect the need for CD40L to induce Fas on self-reactive B cells.<sup>22</sup> The monogenic autoimmune disorders listed above are clinically distinct from the common forms of autoimmune disease, but they illustrate the essential and non-redundant role of tolerogenic costimuli as brakes on autoimmunity. Common autoimmune disorders probably arise from collections of more subtle gene variants that collectively diminish the same tolerogenic pathways. In support of this notion, the type 1 diabetes susceptibility gene in the NOD mouse, *Idd3*, seems to be a variant form of IL-2 that may reduce the in-vivo efficacy of this tolerogenic costimulus.<sup>23</sup>

### Too much immunogenic costimuli

There are many artificially engineered animal models where overexpression of immunogenic costimuli predisposes to autoimmune disease. For example, mice that overexpress TNF $\alpha$ , B7.1, IL-2, or IL-4 on pancreatic islet  $\beta$ -cells are predisposed to type 1 diabetes.<sup>24</sup> Cell death by necrosis releases antigens complexed with immunogenic costimuli, notably the heat-shock proteins HSP70 and HSP96, and necrotic cells activate dendritic cells. An increase in these tolerogenic immunogenic costimuli might explain the immunogenicity of dysplastic tumours that are commonly manifested by the appearance of subclinical autoantibodies to tumour antigens and by paraneoplastic autoimmune syndromes. Along similar lines, the inability to clear dead cells or chromatin might provoke systemic lupus in people with complement C1q deficiency.<sup>14</sup>

### Targets for current and future therapy of autoimmune disease

The unfolding of the human genome project will accelerate assembly of a molecular map of immunogenic and tolerogenic signalling pathways. Translating this knowledge into cures for common autoimmune diseases will involve researchers addressing two key challenges. First, we must develop ways to diagnose the underlying cause of autoimmune disease in individual patients. There is probably little to be gained by giving an exogenous source of tolerogenic costimuli such as TGF- $\beta$  or Fas-ligand to patients with an underlying problem further downstream in the receptors or signal-transduction pathways for these molecules. Methods for obtaining a genetic fingerprint of thousands of immunologically relevant genes will soon become available, and these might provide a way to shortlist the likely pathogenic deficits in individuals. Confirmation will probably require diagnostic biomarkers or specific assays for discrete immunogenic or tolerogenic pathways that can be done on blood samples.

The second critical element is development of protein or small molecule therapeutics that target critical pathways, either augmenting tolerogenic pathways or blocking immunogenic ones. Some of the best current agents for treating systemic autoimmune diseases, such as glucocorticoids, chloroquine, and gold compounds, seem to work by blocking the immunogenic NF $\kappa$ B

pathway.<sup>25</sup> Improvements on these agents depend on narrowing the action to specific subsets of lymphocytes and avoiding the undesirable metabolic effects of glucocorticoids. To cure fully developed autoimmunity, drug targets will need to come from understanding why memory T and B cells are more refractory to tolerogenic signals and why they are less dependent upon immunogenic costimuli.

Engineered proteins and antibodies aimed at blocking specific immunogenic costimuli upstream of NF $\kappa$ B, notably antibodies against TNF $\alpha$ ,<sup>26</sup> CD40L,<sup>27</sup> and the blockers of B7 ligands of CD28, have shown great promise in mouse models and in clinical trials as agents to treat rheumatoid arthritis or establish transplantation tolerance. These strategies may be most effective in individuals with healthy tolerogenic signalling, such as patients undergoing organ transplantation, where the underlying defect is known to be an excess of immunogenic antigen and immunogenic costimuli. In this case, temporarily blocking the immunogenic signals selectively should allow tolerogenic antigen and costimuli to establish an active, reinforcing state of tolerance that persists when blocking therapy is stopped. However, if inherited deficits in tolerogenic signalling prevent restoration of tolerance during a brief window of blocking therapy, it will be necessary to continue the immunogenic blockers for long periods, even though there are many complications associated with long-term immunosuppression.

An attractive notion is the idea of so-called negative vaccines; vaccines that could deliver specific antigens in a way that augments tolerogenic rather than immunogenic signalling. In animal models, delivering low amounts of antigen by the mucosal route, either ingested or nasally, can act as a potent tolerogen. This process might perhaps work by linking the antigen with the tolerogenic costimulus, TGF- $\beta$ , which features in mucosal immune responses.<sup>28,29</sup> The first clinical trial of oral tolerance was unsuccessful, pointing to the need to understand better the mechanisms involved and to develop ways to achieve more reliable linkage between tolerogenic antigen and suitable tolerogenic costimuli. Likewise, rational molecular strategies are needed to improve the success rate of empirical regimes for desensitising allergic reactions to pollens and venoms and to restore tolerance to blood products such as clotting factor VIII.

The one shining example of a successful tolerogenic vaccine is the prevention of erythroblastosis fetalis in Rh-antigen incompatible pregnancies by giving small amounts of anti-RhD antibody. The antibody converts an immunogen (fetal red cells) into a tolerogen by recruiting a tolerogenic costimulus, Fc $\gamma$ R2b. The wide success and cost-benefit of this simple method is an example of how it should become possible to shift the balance back towards tolerance in an antigen specific way for many autoimmune diseases. The key lies in understanding the molecular interplay between immunogenic and tolerogenic pathways and having a way to forge the desired tolerogenic connections in specific lymphocyte clones.

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